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PENETRATION OF ANTIBIOTICS INTO SUBCUTANEOUS TISSUE FLUID WITH SPECIAL
REFERENCE TO A NEW MODIFIED THREAD SAMPLING TECHNIQUE

By

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Malmö 1981

Doctoral thesis at the University of Lund 1981

The English text revised by L. James Brown

Printed in Sweden by
Litos Reprotryck i Malmö AB
Malmö 1981

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I Hoffstedt, B. & Walder, M.
Penetration of ampicillin, doxycycline and gentamicin into interstitial fluid in rabbits and of penicillin V and pivampicillin in humans measured with subcutaneously implanted cotton threads.
Infection 9:7, 1981.
- II Hoffstedt, B. & Walder, M.
The influence of serum protein binding and mode of administration on penetration of five cephalosporins into subcutaneous tissue fluid in human beings.
Accepted for publication in Antimicrobial Agents and Chemotherapy.
- III Hoffstedt, B., Walder, M. & Forsgren, A.
Comparison of skin blisters and implanted cotton threads to evaluate antibiotic tissue concentrations.
Accepted for publication in Journal of Clinical Microbiology.
- IV Hoffstedt, B. & Walder, M.
Penetration of ceftazidime into extracellular fluid in patients.
Journal of Antimicrobial Chemotherapy (In press) 1981.
- V Hoffstedt, B. & Walder, M.
Penetration of fosfomycin and gentamicin into the interstitial fluid.
(Submitted for publication; presented at the 12th ICC in Florence July, 1981).

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INTRODUCTION

Ever since the advent of antibiotics their diffusion into extravascular compartments has been the subject of extensive research, such diffusion being necessary for transport of the drug to the site of infection (8, 21, 23, 27, 58, 60). The importance of this problem is obvious in bacterial meningitis, for example. As known, penetration of antibiotics into the cerebrospinal fluid is under the influence of many factors, such as lipid solubility, ionization, pH-gradient, protein binding, molecular weight, active transport mechanisms and finally, but probably most of all, the presence of inflammation of the meninges, which impairs diffusion of e.g. most β -lactam antibiotics into the cerebrospinal fluid (40).

Antibiotic distribution from the blood stream is influenced by different factors, which vary from compartment to compartment in the body. It is therefore difficult to make any sweeping conclusions. In addition, it is obviously difficult to obtain serial samples of certain fluids, such as cerebrospinal or interstitial fluid, from a given individual, and mean values of single samples from different subjects are not reliable (2, 26).

Antibiotic assay of extravascular fluids in humans raises ethical and technical problems. However, specimens are rarely available, except of sputum and pus.

Several variables are known to influence the distribution of antibiotics in the body e.g. degree of serum protein binding, degree of dissociation, lipid solubility and molecular weight (9). The influence of these factors on the penetration of antibiotics into extravascular compartments differs from one part of the body to another. Thus, serum protein binding is the most important factor determining the distribution to parts requiring passage of the drug only through the pores of the capillary endothelium, "shallow compartments" (32, 37). Such parts are interstitial fluid and highly vascularized organs. Diffusion to other parts in the body, where no capillary pores exist, "deep compartments", such as the central nervous system, the interior of the eye and the prostatic gland depends largely on the lipid solubility of the drug (3, 37).

A wide variety of experimental techniques have been used in the investigation of the distribution of antibiotics in extravascular compartments.

In 1963 Guyton measured the interstitial fluid pressure in dogs (30). It was assumed that the fluid entering the capsules would have the same properties as true interstitial fluid and be in direct communication with other body fluids (11). The first to use a similar technique for assaying antibiotic levels was Waterman & Kastan (59). Their report prompted other researchers to study the penetration of different antibiotics into the fluid in such capsules or cages (12, 16, 51). Different collecting containers were developed in an effort to achieve as large an area of diffusion as possible per unit of volume of the container.

In 1971 Raeburn placed filter paper discs on a damaged area of the skin and measured antibiotic levels in the inflammatory exudate (45). The same technique was later used by Gillett & Wise in a study on the penetration of four cephalosporins (29).

In 1972 Tan et al had used a saline-filled chamber with a surface area of 1 cm^2 (56). They placed it on the skin where the epidermis had been destroyed. Antibiotic levels in these chambers varied closely with the serum levels.

In 1974 Barza & Weinstein published their study on antibiotic penetration into fibrin clots in vivo (4). In that study, however, they measured antibiotic levels not only in fibrin clots, but also in interstitial fluid, which they collected in subcutaneously implanted filter paper discs. The concentration time course of the drugs in fibrin clots differed from that in filter paper disc fluid, the concentration time course in the clots resembling those in subcutaneous cages; those in the filter disc fluid, the serum concentration time course.

In 1976 Simon et al presented their technique using skin blisters induced by cantharidin 12 hours before administration of the antibiotic (55).

In 1976 Kiistala & Mustakallio presented their technique with suction blisters (36). Their method was later used by Schreiner et al who produced suction blisters 12 hours before the administration of the antibiotics (52).

In 1979 Ryan published a simple method for collecting interstitial fluid with subcutaneously implanted cotton threads (49).

An elegant method for sampling true interstitial fluid has been devised by Haljamäe (31). However, the amounts of fluid obtained with this method are too small to permit assay of antibiotic concentrations.

It is obvious that for ethical or technical reasons many of the above methods cannot be used routinely in humans. This applies particularly to subcutaneous implantation of cages and fibrin clots. Moreover, the results obtained with these techniques do not reflect the concentration time course of the antimicrobials in interstitial fluid, but rather in that of a deep compartment (50).

AIMS OF THE STUDY

The purpose was to devise a simple method for obtaining serial samples of human subcutaneous tissue fluid in one and same patient for determining the concentration of antibiotics in such fluids in patients receiving chemotherapy.

It was decided to try to modify the method of subcutaneous implantation of cotton threads in such a way that it would:

- be technically simple and safe and produce samples for microbiological assay;
- reflect the antibiotic levels in tissue fluid without any undue inflammatory reaction;
- give reproducible results, and
- be so well tolerated by the human body as to warrant its use in clinical studies.

With this purpose in mind we carried out the following investigations.

1. Comparison of the method with another widely used technique.
2. Estimation of the influence of serum protein binding on penetration of antibiotics into the interstitial fluid.
3. Comparison of levels of antibiotics in the interstitial fluid following intravenous injection and intravenous infusion.
4. Appraisal of the penetration of antibiotics into the interstitial fluid for any variation with molecular weight.

5. Determination of steady state levels of antibiotics in the interstitial fluid in the absence and in the presence of varying severity of impairment of renal function.

METHOD (I)

Interstitial fluid was sampled with cotton threads (umbilical tape) manufactured by Ethicon. We chose a cotton thread because it is very absorbent and homogenous. After having tested different types of needles we decided to use a non-traumatic needle attached direct to the umbilical tape in order to keep tissue damage and bleeding to a minimum. The threads were manufactured by Ethicon at our request with a thread 20 cm long attached to the needle, and each needle was delivered sterilized in a separate envelope. From each thread six to seven samples could be obtained (Fig. 1).

After disinfection of the skin and subcutaneous injection of 2-3 ml of a local anaesthetic, Xylocain® (10 mg/ml), the threads were implanted in the ventral surface of the forearm of the volunteer or patient (Fig. 2). Two threads were always implanted in order to obtain paired samples at each sampling time. The first 1-2 cm of each thread was inserted subcutaneously. After 30-60 minutes the antibiotic was administered and after varying intervals the serum and thread samples were taken simultaneously. The subcutaneous part of the thread was pulled out and cut off. At the same time a new piece of the same thread was pulled in subcutaneously to obtain another sample. Pieces of thread that had been cut off were placed separately in small tubes and stored at -20°C together with the serum samples until assayed. The average amount of fluid absorbed by each piece of thread cut off was $4.5 \mu\text{g/ml}$ i.e. 58 % of its original weight.

The maximum blood content of an analysed thread was 5 g/l measured with a spectrophotometer after elution of the fluid absorbed, but most of the threads analysed were not contaminated with blood. Thus, blood contamination was no problem.

To check whether the small amount of the anaesthetic around the thread had any demonstrable effect on the absorption of the drug, the concentration of the latter was measured in a freshly inserted thread and in one that had been in position for at least 2 hours. In that experiment we also measured the time necessary for the concentration of the drug in a freshly implanted thread to reach equilibrium with that in the surrounding interstitial fluid. Two threads



Fig. 1. A cotton thread with attached autotraumatic needle.



Fig. 2. The forearm of a human volunteer with two cotton threads implanted.

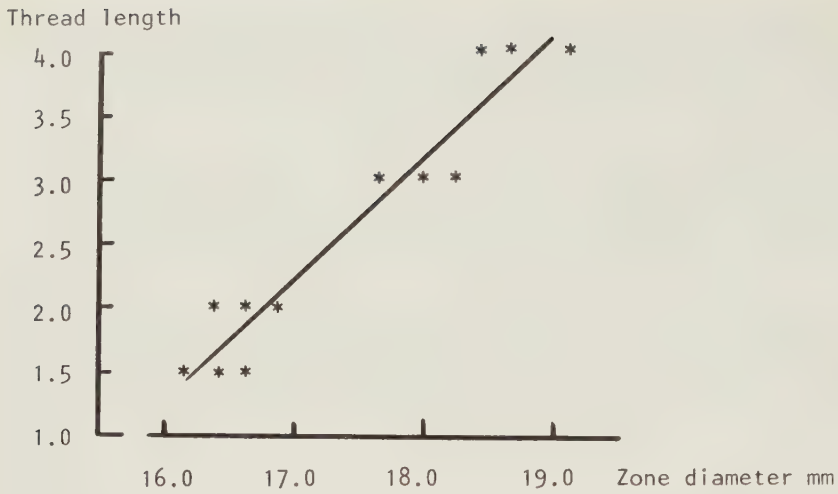


Fig. 3. Relation between thread length and obtained zone diameters in an agar plate.

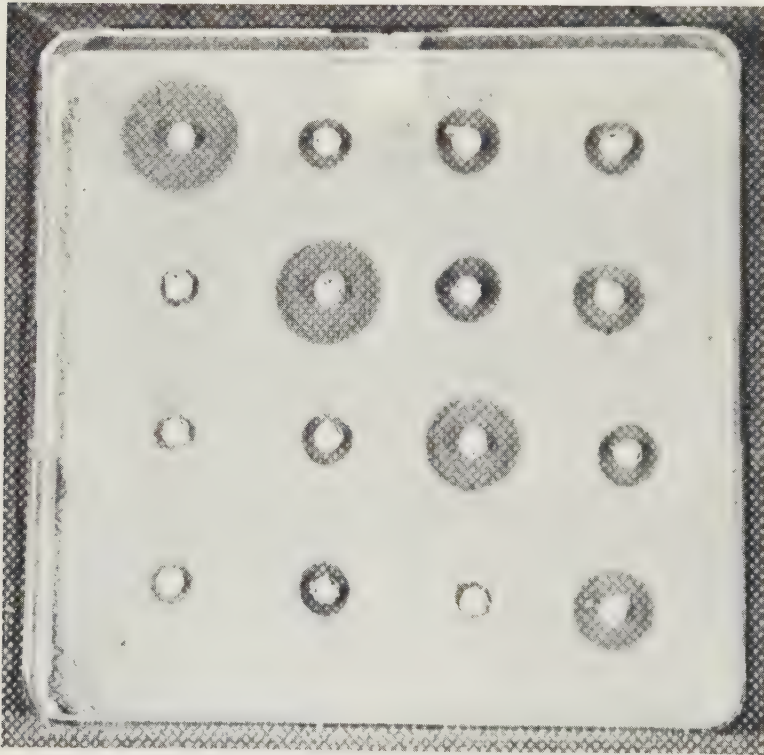


Fig. 4. A disposable plastic assay plate with cotton threads.

were implanted subcutaneously in the back of a rabbit. At least 2 hours later they were sampled after further intervals of 1, 2, 5, 10, and 30 minutes. The antibiotic levels in these threads were then compared with those freshly implanted. It is clear from Table I that equilibration required at most 2 minutes and that the local anaesthetic had no demonstrable effect on the rate of absorption of the drug.

To ascertain whether the concentrations found varied with the length of the threads and hence with the amount fluid accumulated in them, cut pieces of different length were impregnated with antibiotic solution of known concentration. As can be seen from Figure 3 the levels were proportional to the length of the thread and thus to the amount of fluid absorbed by them.

Table I. Obtained zones in an agar plate for threads newly implanted and implanted 2 hrs earlier.

Time after implantation	Zone, mm (Newly implanted)		Zone, mm (Implanted 2 hrs earlier)	
	Thread A	Thread B	Thread C	Thread D
1	17.8	15.9	18.1	17.6
2	18.2	18.5	18.5	18.1
5	18.1	17.9	18.8	18.3
10	17.8	18.0	17.9	17.8
30	18.3	18.1	18.6	18.2

The effect of leaving threads in the tissue for 24 hours before administration of the drug was studied in one human volunteer. By the end of this period there was a moderate inflammatory reaction around the threads with redness and local oedema. The fluid in the threads was no longer water clear as in the previous experimental threads, but yellowish. The threads were easily withdrawn, but they were stiff and hard and difficult to cut into pieces for antibiotic assay. As expected, the antibiotic levels in the 24-hour threads were much higher than in any of the others (Table II).

To find out whether the method used for elution of antibiotics from the threads was accurate 1 cm long pieces of thread were impregnated with different known concentrations of antibiotic solutions and then assayed on an agar plate, as described below. The levels found were compared with known concentrations of pure antibiotic solutions on the same agar plate. The calculated "regression lines" paralleled each other, but the concentrations found in the threads were lower than those in the pure solutions. This indicated that the elution was not complete. To avoid this error threads impregnated with antibiotic solutions of known concentrations were used as a standard.

Bacteriological method

The antibiotic concentrations of the threads were determined with an agar wells diffusion technique. A piece of thread 1 cm long was cut into smaller pieces which were placed in an 8 mm well in an agar plate, after which 100 μ l of buffer was added to each well to insure an even distribution of the antibiotic in the agar. The agar used most frequently was DST 125 ml in 12" x 12"

Table II. Obtained antibiotic concentrations in threads implanted 1 hour and 24 hrs before the antibiotic administration.

Experiment	Concentration μ g/ml	
	"New thread"	"Old thread"
A	2.9	7.3
B	3.8	9.7
C	2.1	4.9

disposable plastic assay plates (Fig. 4). Pieces of sterilized thread, 1 cm long, and impregnated with different concentrations of antibiotic solutions served as antibiotic standards for the threads.

Severe contamination of the threads with blood led to false low values if the drug being tested was strongly bound to serum proteins. However, if the bond was weak, the influence of the contamination was negligible.

The serum concentrations were measured with the conventional agar well diffusion technique.

Tolerance

Both human volunteers and patients tolerated the implanted threads very well. Only withdrawal of the last pieces of the threads caused any pain, which was only mild. No post-experimental infections were seen. The wounds healed completely within a week and left only slight dyspigmentation. No scars were observed.

COMPARISON BETWEEN THE THREAD TECHNIQUE AND THE SUCTION BLISTER TECHNIQUE (III)

Our method with subcutaneously implanted cotton threads for collecting interstitial fluid was compared with an established method. We chose the suction blister technique because of our earlier experience in the development of blisters by suction and because many recently published papers refer to that method (24, 52).

Six male volunteers took part in the study. Ampicillin was given i.v. in a dose of 2 g and cloxacillin in a dose of 1 g. All the volunteers received both antibiotics with an intermission of at least one week.

Skin blisters were produced by tightly strapping a perspex plate with eight holes against the volar surface of one forearm.

A negative pressure was applied by a suction pump for one hour and a half. Distal to the perspex plate on the lateral aspect of the forearm two cotton threads were implanted subcutaneously in the way described earlier.

Samples were obtained of the blood, blisters, and threads 15, 45, 90, 150, 240, and 300 minutes after the infusion had been started.

The total concentrations of the antibiotics in serum, blister fluid, and thread fluid were measured with a microbiological method. All samples were assayed in duplicate.

The protein in the blister fluid and in the thread fluid was quantified with a modified Manchini technique by Dr A Grubb, Department of Clinical Chemistry, Malmö General Hospital.

The penetration of cloxacillin into both the thread fluid and the blister fluid was delayed and reached a maximum level after 45-90 minutes. In contrast, ampicillin promptly entered both extracellular fluids. The half-life of ampicillin as well as that of cloxacillin in blister fluid was prolonged compared with that in serum and in thread fluid. However, the half-lives of both antibiotics in the thread fluid were approximately the same as in serum.

The ratio between peak levels in the thread/blister fluid and serum was very low for cloxacillin compared with that for ampicillin. The penetration of ampicillin into the thread fluid calculated as the area under the curve for the thread fluid level divided by the area under the serum curve multiplied by 100 was 26 % and that of cloxacillin, 12 %. The penetration of ampicillin into blister fluid was 68 %. The thread fluid levels of both cloxacillin and ampicillin almost paralleled the serum levels, indicating a fast dynamic equilibrium between antibiotics in the blood and in thread fluid. Hence the thread fluid levels presumably reflect those in the interstitial fluid. This possibility was strengthened by chemical analysis of the thread fluid.

The albumin content of the blister fluid was 19.3 ± 3.9 g/l, that of the thread fluid 16.0 ± 1.0 g/l, and that of serum 45.3 ± 2.5 g/l.

The antibiotic in the blister fluid, on the other hand, was characterized by a prolonged elimination time, and after a couple of hours the blister fluid level exceeded the serum levels. This slow elimination could be explained by the volume of the blister being relatively large compared with the surface through which the drug could pass.

The difference in antibiotic pharmacokinetics of the blister fluid and

the thread fluid were larger for cloxacillin, a drug binding a large amount of serum protein. The peak blister fluid levels of cloxacillin were much lower than those in the thread fluid. Even the time required by the drug to reach its peak level was delayed. This indicated the existence of a factor limiting e.g. protein binding, the penetration, and elimination of cloxacillin.

One reason why the cloxacillin levels in the blister fluid were low may be the time required for achievement of a balance between the serum and blister concentrations with consequent shortening of the time for the drug to penetrate into the large blister fluid compartment before the serum level falls. The slower elimination of cloxacillin from the blister fluid might also be due to the binding of cloxacillin to extracellular fluid proteins (18, 19).

The levels of ampicillin in the blister fluid exceeded those in the thread fluid. This difference might partly be explained by the greater tissue damage caused by the blister technique, as indicated by higher albumin levels in the blister fluid.

The observations made in this study appear to warrant the conclusion that the thread technique reflects the kinetics of antimicrobials in extracellular fluid, where the degree of penetration into the extracellular fluid depends largely on the degree of protein binding of the drug. On the other hand, the blister technique with blisters made before the administration of the drugs reflects the situation in a deep compartment resembling that in different subcutaneously implanted cages and fibrin clots.

THE INFLUENCE OF SERUM PROTEIN BINDING AND MODE OF ADMINISTRATION ON PENETRATION OF FIVE CEPHALOSPORINS INTO SUBCUTANEOUS TISSUE FLUID IN HUMAN BEINGS (II)

In the investigation of the influence of serum protein binding of antibiotics and their penetration into interstitial fluid in humans we used five cephalosporins. Cephalosporins were chosen because they closely resemble one another in molecular weight, degree of lipid solubility and dissociation at physiological pH. However, they differ widely in degree of serum protein binding.

Another question to be answered was whether the penetration of these agents varied with the method of administration i.e. as i.v. injections and infusions. The antibiotics used were cephradine (serum protein binding capacity of 10 %), cefuroxime (33 %), cefotaxime (40 %), cefoxitin (70 %), and cefoperazone (90 %).

Each drug was tested as an injection and as an infusion on three to four healthy male and female volunteers. Each subject received one intravenous injection for five minutes and after at least one week an intravenous infusion for 30 minutes.

Interstitial fluid was sampled with subcutaneously implanted cotton threads. The half-life of the drug in serum and in interstitial fluid was calculated from the serum and interstitial fluid concentrations, as was the penetration (calculated as area under the interstitial fluid level curve divided by the area under the serum level curve multiplied by 100) and the ratio between the peak interstitial fluid level and the peak serum level and finally the period when the antibiotic levels in interstitial fluid were above 2 µg/ml.

It was found that the degree of penetration into interstitial fluid varied inversely with the degree of serum protein binding of the antibiotic. The correlation coefficient was -0.97. The penetration calculated as above varied between 9.6 % (cefoperazone) to 47.0 % (cephradine). The interstitial fluid level curves almost paralleled the serum curves for all drugs except for cefoperazone, which showed a longer half-life in interstitial fluid. The close similarity between the serum and interstitial fluid curves suggested that the transport of a drug to and from interstitial fluid occurs mainly by free diffusion. The prolonged half-life of cefoperazone could be explained by its high protein binding capacity and consequent binding to interstitial fluid proteins creating a "depot" like effect that also have been reported for other drugs (1, 33).

The influence of the mode of administration on the antibiotic levels in the interstitial fluid was not evident since both the absolute levels in the interstitial fluid and the area under the interstitial fluid level curves were not statistically significant different whether the drugs were given as injections or infusions.

It would thus appear that penetration of an antibiotic into interstitial fluid varies inversely with the serum protein binding of the drug. No difference in interstitial fluid levels were seen whether the drug was given by infusion for 30 minutes or by injection. The choice between the two methods in a given case must therefore be decided by such factors as the level of the threshold of pain, risk of thrombophlebitis and practical factors.

PENETRATION OF FOSFOMYCIN AND GENTAMICIN INTO INTERSTITIAL FLUID IN HUMAN BEINGS (IV)

In this study, we compared the penetration of fosfomycin with that of gentamicin into interstitial fluid in human beings. At therapeutic serum levels neither fosfomycin nor gentamicin is believed to bind serum protein to any notable extent, but they differ in molecular weight (gentamicin 545 and fosfomycin 138).

1 g fosfomycin was injected intravenously for five minutes to three volunteers and 120 mg gentamicin to three others. Multiple serum and thread samples were taken thereafter.

The half-life of fosfomycin in serum was 2 hours; that of gentamicin 1.5 hours, corresponding values in interstitial fluid were 2 hours and 2.5 hours.

The penetration, calculated as the area under the interstitial fluid level curve divided by that under the serum level curve multiplied by 100 was $36.6 \% \pm 11.7 \%$ for fosfomycin and $66.8 \% \pm 13.2 \%$ for gentamicin.

However, the peak interstitial fluid level was $2.4 \mu\text{g/ml}$ for gentamicin and $20.0 \mu\text{g/ml}$ for fosfomycin.

Judging from the above observations, then, the diffusion of antimicrobials into interstitial fluid varies but little with their molecular weights.

PENETRATION OF CEFTAZIDIME INTO EXTRACELLULAR FLUID IN PATIENTS (V)

This study of the penetration of ceftazidime into extracellular fluid was carried out in 8 patients receiving treatment with ceftazidime. Creatinine clearance was calculated by a method described by Siersbaek-Nielsen et al

(53). The patients were divided into two groups according to their creatinine clearance: one with normal clearance ($\geq 60 \text{ ml/min} \times 1.73 \text{ m}^2$) and one with renal insufficiency and impaired clearance ($< 60 \text{ ml/min} \times 1.73 \text{ m}^2$).

Each group consisted of 4 patients treated with 1 g ceftazidime three times a day in the form of short bolus injections for five minutes.

Samples of the blood and extracellular fluid were obtained as described earlier. The samples were obtained after treatment for at least three days, by which time patients were presumably in a steady state.

The penetration of ceftazidime, calculated as the area under the extracellular fluid level curve divided by that under the serum level curve multiplied by 100 was equal to or better than that of other cephalosporins. In the patients with normal renal function the half-life of ceftazidime was 2.2 hours in the extracellular fluid and 2.5 hours in the serum. The corresponding values in patients with renal insufficiency were 11.5 and 7.9 hours. The quotients between the peak levels in the extracellular fluid and serum and between the area under the extracellular fluid level curve and that under the serum level curve were around 50 % in each group. On the other hand, the area under the extracellular fluid curve and that area under the serum level curve was twice as high in the presence as in the absence of renal insufficiency.

The maximum levels of ceftazidime in both extracellular fluid and serum were well above the minimum inhibitory concentrations for the pathogens susceptible to the drug. It would thus seem that a dose of 1 g per injection should be adequate.

The rather high initial levels in the extracellular fluid suggest that ceftazidime might be effective in the treatment of infections not only of the blood and urine, but also of soft tissues.

The observations made in this study suggest that the half-life of ceftazidime in serum in patients is longer than that found in healthy volunteers (14). The prolonged half-life in connection with high extracellular fluid levels and serum levels even five hours after injection of the drug suggest that two doses a day might be sufficient in the treatment of most patients.

GENERAL DISCUSSION

One of the most important prerequisites for therapeutic success in anti-microbial therapy is that the substance reaches the site of infection and achieves an appropriate level there.

But this is not the only reason for studying antibiotic levels in the extracellular fluid. First, it constitutes about 25 % of the body weight and hence is a large compartment. Of the extracellular fluid, approximately 20 % is plasma and about 50 % interstitial fluid, corresponding to 8.4 l in an adult male (39). Second, many infections start and proceed in this fluid. Third, when antibiotics are used prophylactically in surgery, they must reach adequate levels in the extracellular fluid since many surgical infections spread via it. Finally, it must also be important to know after how long appropriate levels in this fluid persist after the antibiotic had been administered. Many experimental methods have therefore been devised to obtain interstitial fluid, as described in the introduction.

One theoretical model for estimating the distribution of drugs in the body is to calculate the apparent volume of distribution. This is an indirect method that gives a rough estimation of the distribution of the drug, but it teaches nothing about where in the body the antibiotic is to be found, but only in what volume the antibiotic has been dissolved.

A theoretical method for predicting the antibiotic distribution has been presented by Cronberg (20).

As it is known that the tissue fluid is not homogenous but represents a large amount of fluid readily available to most antibiotics and in more or less equilibrium with other compartments of the body, direct methods for evaluating antibiotic concentrations in tissue fluid are desirable.

Subcutaneously implanted cages

The concentration time course of antibiotics in subcutaneously implanted cages is characterized by a slow penetration of the antimicrobials, delayed peak levels and slow elimination from the cages compared with the course of the serum levels (8, 12, 14, 16, 34, 57, 59, 61). This antibiotic time course contrasts sharply with what we found in the fluid obtained by subcutaneously

implanted cotton threads, i.e. a course almost parallel to that of the serum levels. Three tentative explanations can be offered for this difference. First, the cages are covered by a granulomatous fibrin layer like an abscess, a layer hindering free direct diffusion of the antibiotics from the capillaries to the cage fluid (50). Second, the volume of the cages is very large compared with the area of the surface through which the drug can pass (8). Third, the albumin content of the cage fluid is high (11, 16). As pointed out by Peterson & Gerding and Cars et al, the protein content of extravascular fluid greatly influences the achieved levels of especially highly protein bound drugs (14, 43). In summary, the antibiotic time course in the subcutaneously implanted cages closely resembles that of an abscess and does not reflect the conditions in interstitial fluid (34, 50).

Subcutaneously implanted fibrin clots

This method was first described by Weinstein et al and was used by Barza in several studies of antibiotic penetration (60, 4, 5, 6). The antibiotic time course in the fibrin clots resembled that in an abscess and consequently that of a subcutaneously implanted chamber. The half-life of the drug is much longer and its peak level much later in fibrin clots than in serum. The slow elimination of antibiotics from them and the delayed peak levels in them could be explained by binding of the drug to them and a comparatively large volume of them. Summing up, fibrin clots do not reflect antibiotic levels in the interstitial fluid, but in a deep compartment.

Subcutaneously implanted filter paper discs

This method was also used by Barza & Weinstein in rabbits and yielded results similar to those obtained by us with subcutaneously implanted cotton threads (4). This was because of the smallness of their volume and their closeness to the capillaries. Probably the fluid accumulated in them had the same chemical properties as the fluid obtained in cotton threads.

Skin chambers

This method used by Tan et al gave an antibiotic concentration time course almost parallel to the serum concentration time course, but with a delayed peak level (56). The protein content of this chamber fluid was very low, on the average only 1.5 g/l (maximum 4.5 g/l), which might explain why he did not find any correlation between the antibiotic levels in the fluid and the

protein content. Frongillo et al did not find any differences in fluid antibiotic level when the chamber was filled with serum instead of saline (25). This contrasts with what Peterson & Gerding reported in 1978 (43). They found the level of cephazolin to vary with the protein concentration in the subcutaneous chamber fluid. The concentrations of the antibiotics in the chamber fluid are lower than those in skin blister fluid, for example, and might be explained by a dilution process of the dermal base exudate (25).

The results obtained with this technique resemble those we found with our method and thus reflect the conditions in a shallow compartment.

Skin windows

This method was originally used by Raeburn in 1971, who found the antibiotic levels in fluid from the skin windows to be close to those in the serum (45). His results have been confirmed by Frongillo et al (25). The disadvantages of this method were that it took the filter paper disc about one hour after implantation to absorb sufficient fluid to permit antibiotic assay and that the results obtained were influenced by the point of time when the discs were applied to the abraded skin. However, the method must be regarded as one showing the conditions in a shallow compartment.

Skin blisters

This method was originally described by Kiistala & Mustakallio in 1967 (36). The blisters could be induced mainly in two ways; by suction as originally described or by cantharidin (25, 52, 54, 62).

The fluid obtained from these blisters was one of the few artificial extracellular fluids that have been chemically analysed by several researchers. The protein content varies with the severity of the trauma caused by the induction of the blisters. Simon et al found a protein content of 55-50 g/l in the fluid obtained, Schreiner et al 45 g/l, Frongillo et al 41-32 g/l, Bruun et al 30 g/l, and we found 30-25 g/l (10, 25, 52, 55). As mentioned before, this varying protein content has a great impact on the results obtained, especially for strongly protein bound drugs (43).

We tried to avoid unnecessary tissue damage when developing the blisters by using a smaller negative pressure during the first hour than during the

last half hour of suction. That this was a more atraumatic way to produce blisters was apparent from the protein content of the blister fluid.

The findings varied with the age of the blisters before administration of the antibiotic. We used freshly produced blisters, but most authors have used 12-hour old blisters (25, 52, 62). As shown by Frongillo et al, cefotaxime penetrates faster and the antibiotic levels in the blister fluid are higher in freshly produced blisters (25).

In our study we tried to elucidate the blister technique by using two antibiotics (ampicillin 20 % and cloxacillin 94 %) differing widely in protein binding capacity.

We found a delay of the peak values and a prolonged elimination of the drugs in the blisters. The elimination of cloxacillin was delayed most indicating its greater binding to extracellular fluid proteins (19).

Also the levels of cloxacillin in the blister fluid were very low, probably because of the time required for equilibration between serum antibiotic and antibiotic in the already developed blisters. The time for cloxacillin to penetrate into the blister fluid before the serum level begins to fall was then too short (48).

The results we obtained with the suction blister technique showed that they did not reflect what happened in true interstitial fluid, but rather the situation in subcutaneously implanted cages, fibrin clots and other deep compartments. The results varied also with the way the blisters were produced and thereby with the extent of tissue damage. We therefore think it necessary to determine the protein content of the blister fluid as an indicator of the tissue damage in the interpretation of values obtained with this method.

The blister technique can be used without difficulty in human volunteers, but as it requires attachment of the suction pump to the body for at least 1½ hour it may be difficult to use on patients.

Subcutaneously implanted cotton threads

This method, first described by Ryan in 1979 and modified by Hoffstedt & Walder (1980 and 1981), has many advantages (49). In this method the anti-

biotic time course of both low- and high-protein bound antibiotics run fairly parallel to the serum antibiotic time course. Chemical analysis of the fluid samples shows only minimal tissue damage and inflammatory reaction, as indicated by the total protein and albumin content. This implies that the fluid studied with this method originated from a shallow compartment and is virtually interstitial fluid. The method also permits serial samples and, if desired, in duplicate or triplicate.

The absolute antibiotic levels in interstitial fluid in our studies were lower than those found by Ryan. This could be explained by the technique used by him. Ryan implanted the threads 24 hours before administration of the antibiotic and thereby induced an inflammatory reaction around the threads with consequent leakage of protein from the capillaries into the threads. Furthermore, he used several threads to obtain multiple thread samples and thereby produced a stronger reaction than we with only two threads. This assumption was confirmed by our finding that threads implanted 24 hours before administration of antibiotics yielded much higher antibiotic levels than newly implanted ones.

In conclusion, the method using two subcutaneously implanted cotton threads is probably the best hitherto available method for collecting interstitial fluid for antibiotic assay in human beings. The advantages of this method are as follows:

1. The samples obtained reflect the antibiotic levels in the interstitial subcutaneous tissue fluid.
2. The technique is simple and gives reproducible results.
3. The method is well tolerated and can be applied to healthy volunteers and to patients.

Influence of serum protein binding on penetration of antibiotics into interstitial fluid

Some authors have demonstrated that strongly serum protein bound drugs yield an inferior penetration into extravascular foci than low protein bound drugs (5, 7, 13, 14, 17, 35). As only the free unbound drug is

available for antibacterial activity and for distribution into extravascular compartments, a drug with a low degree of serum protein binding is supposed to be better in the treatment of infections outside the bloodstream (35, 37, 47, 48, 56). However, except for the studies by Tan et al and by Howell et al these experiments have been performed on animals (35, 56). In the studies in humans Tan et al found the penetration of antibiotics into interstitial fluid to vary with the degree of serum protein binding (56). However, Gillet & Wise could not corroborate their finding (29). This might have been because their technique caused more tissue damage with the result that the tissue fluid obtained was very similar to serum. However, in a recent paper Wise et al found a linear correlation between protein binding of the antibiotics and their penetration into blister fluid, as calculated from the area under the curve of the protein-free fraction (62).

In our study of penetration of five cephalosporins into interstitial fluid we found a close correlation between serum protein binding and penetration. Thus, it is the free fraction that diffuses into tissue fluid and it is in equilibrium between plasma and tissue fluid.

Variation of antibiotic penetration into interstitial fluid with the method of administration

In 1953 Eagle demonstrated that in the treatment of streptococcal infection in mice a continuous supply of penicillin accelerates control of the infection (22). Barza et al, on the other hand, found strikingly higher levels of ampicillin in subcutaneous fibrin clots when the drug was injected intermittently than when it was given by continuous infusion (6, 7). These findings were not corroborated by Carbon et al, who demonstrated significantly higher levels of cephalothin in a subcutaneous siliastic tissue cage fluid after infusion for 0.25 hour than after intramuscular injection or infusion for 1 hour (13).

Using subcutaneous chambers in the rabbit Peterson et al found that the cefazolin concentrations in the chambers were significantly higher after a continuous infusion than after intramuscular injections of the drug (44).

In a recent review, Kunin regarded intermittent dosage superior to continuous administration of an antibiotic (38).

In our study, in which we used only a single dose, we found no difference in interstitial fluid levels or penetration of the drug into the interstitial fluid after a short bolus injection and 0.5 hour infusion of the same amount of antibiotics.

Variation of the penetration of antibiotics into the interstitial fluid with the molecular weight of the drugs

The literature contains but little about the effect of molecular weight upon antibiotic penetration into the interstitial fluid. However, as most of the drugs used have a molecular weight of about 300-500 i.e. small enough to pass through the capillary pores, differences in this weight range need not be expected to have any effect on penetration (17, 32, 42). We compared the penetration of fosfomycin (MW 138) and gentamicin (MW 543) into the interstitial fluid. It was assumed that neither drug was bound to serum proteins at the serum levels achieved.

The penetration of gentamicin into interstitial fluid was 67 % and that of fosfomycin 37 %, calculated from the area under the interstitial fluid level curve divided by the area under the serum level curve $\times 100$. Thus, the larger molecule showed the better penetration. This might be due to other physical properties of the molecules.

Influence of impaired renal function on penetration of ceftazidime into the interstitial fluid

Only few studies are available of the extravascular levels of antibiotics in the presence of impairment of renal function. Gibaldi & Schwartz found that the volume of distribution of drugs with a short half-life and mainly renal route of excretion, was markedly reduced when probenecid was administered concurrently (28). We, however, found the area under the interstitial fluid level curve and that under the serum level curve to be increased when renal function was impaired. Renal dysfunction had no demonstrable effect on penetration of the administered drugs into the interstitial fluid when the penetration was calculated as the quotient between the area under the interstitial fluid level curve and that under the serum level curve.

It is also well-known that the degree of serum protein binding diminishes in uraemia (18).

The interstitial fluid levels almost paralleled the serum levels even when renal function was impaired. These findings agree to those made by Cars et al in nephrectomized rabbits (14).

Thus, differences in renal function affected the penetration of ceftazidime in that the half-life was longer, and the concentrations in serum and tissue fluid, higher in patients with renal insufficiency.

IN CONCLUSION

Determination of tissue fluid concentrations are necessary to establish optimal dosage of an antibiotic, and for drugs used as prophylaxis in surgery it is important to know their actual levels in interstitial fluid and how long after administration, they requires to reach a desired level.

SUMMARY AND CONCLUSIONS

1. A new type of a cotton thread for collecting tissue fluid in subcutaneous tissues was used.
2. A technique was devised for assaying antibiotics obtained with these threads.
3. The results obtained with this method reflect the antibiotic levels in the interstitial subcutaneous tissue fluid and therefore differ from those obtained with the suction blister technique.
4. The technique is simple and precise.
5. The method is well tolerated and can be applied to healthy volunteers and to patients.
6. It was found that the free fraction of an antibiotic diffused into the tissue fluid and reached a state of balance between the plasma and the interstitial fluid.
7. No difference in interstitial fluid levels was found whether the antibiotic was given by intravenous injection for 5 minutes or infusion for 30 minutes.

8. A moderate difference in molecular weight of an antibiotic was found to have but little effect on its penetration to the tissue fluid.
9. Renal insufficiency influences the penetration of an antibiotic in that the half-life is prolonged and the concentrations in the serum and tissue fluid are higher.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to

Associate Professor Stig Cronberg, my present chief at the Department of Infectious Diseases, who introduced me into the present field of research and to whom I am greatly indebted for valuable advice, criticism and continuous encouragement throughout the study;

Professor Arne Forsgren, for providing excellent working conditions at the Department of Clinical Bacteriology and for constructive criticism;

Dr Mats Walder, co-worker and friend, for invaluable help with microbiological problems;

The late Associate Professor Sture Larsson, who initiated my interest in antibiotic distribution in tissue fluids;

Dr Michael Ryan and Glaxo Group Research Limited, England for stimulating collaboration and financial support;

Miss Berit Larsson for skilful technical assistance;

Mrs Agneta Hansson and Mrs Maria Rissler for excellent secretarial help;

The Board of Directors of Malmö General Hospital, for generously granting leave of absence;

All colleagues at the Department of Infectious Diseases for taking over some of my clinical practice, when I devoted myself to research;

All my patient animal and human volunteers, completely necessary for this study;

Glaxo AB, Sweden, Merck Sharp & Dohme AB, Sweden, Pfizer AB, Sweden, Hoechst AB, Sweden, Squibb AB, Sweden, and Dumex AB, Sweden for generous financial support without which this investigation would not have been possible.

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